

THE EFFECT OF DILUTION OF SEA WATER ON THE  
ACTIVITY AND LONGEVITY OF CERTAIN MARINE  
CERCARIE, WITH DESCRIPTIONS OF  
TWO NEW SPECIES

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INTRODUCTION

The present investigation was undertaken to secure data bearing on the question of the origin, distribution, and evolution of present groups of digenetic trematodes. The specific problem under consideration develops from the observation that several families of the digenetic trematodes have representatives in both marine and fresh-water hosts. A brief statement of the problem was given by Stunkard (1930). If the trematodes found in both marine and fresh-water hosts and assigned to common families, and even to common genera, have true phylogenetic relationships; *i.e.*, if they have descended from common ancestors, rather than consisting of groups that through convergence show morphological and developmental similarities, their distribution raises an exceedingly difficult biological problem.

There appears to be little doubt but that the parasites in question are actually closely related. Among the gasterostomes, *Bucephalus polymorphus* von Baer, 1826 was described from fresh-water fishes and *Bucephalus haimeanus* Lacaze-Duthiers, 1854 was described from marine fishes. Tennent (1906) traced the life cycle of the latter species, and the recent studies of Woodhead (1929, 1930) have demonstrated the development of two species that occur in fresh-water hosts. The similarity in structure and development between the marine and fresh-water species is so striking that it strongly indicates close relationship.

In the Prosostomata there are a number of families whose members occur in both marine and fresh-water hosts. The family Aspidogastridae, *e.g.* (see account by Stunkard, 1917), contains species that infest mollusks, fishes and turtles of fresh water, and others that occur abundantly in marine fishes. In this aberrant family, also, the morphological and developmental agreement is too close to be satisfactorily explained on the basis of convergence.

Several other families of the Digenea manifest the same type of

distribution. The family Fasciolidæ (see Stunkard and Alvey, 1930) contains one group of genera which infests the livers of terrestrial herbivores, and another, consisting of *Campula*, *Lecithodesmus*, *Orthosplanchnus*, and *Zalophotrema*, which occurs in the livers of various marine mammals. In the family Parauphlostomidæ there are a large number of genera, most of which parasitize the hoofed mammals, although one genus, *Chiorchis* (see Stunkard, 1929) occurs in the Atlantic manatees. It is of course possible that the sea cows, frequenting the mouths of rivers, acquire these parasites in fresh water. With few exceptions, members of the Heterophyidæ occur in terrestrial vertebrates and the larvæ develop in fresh-water snails, while *Cryptocotyle lingua* infests the intestine of fish-eating birds and the larvæ develop in the marine snail *Littorina littorea* (see Stunkard, 1930a). In the family Pronocephalidæ, several genera have been reported from marine turtles, while one species was described (Stunkard, 1930) from the fresh-water turtle, *Amyda*, and a second has been described by Mackin (1930), from *Pseudemys elegans*. According to Fuhrmann (1928) the family Stenophoridæ (syn. Fellodistomidæ, see Stunkard and Nigrelli, 1930) contains species from the intestine of both marine and fresh-water fishes. Representatives of all of these groups have been studied by the senior author and the results afford cumulative evidence that both marine and fresh-water hosts harbor closely related species of parasites.

Nicoll (1915, 1924) lists other families which have representatives in both marine and fresh-water fishes. Furthermore, there are several genera, e.g., *Azygia* (see Manter, 1926), which have species in both marine and fresh-water hosts. No exhaustive review of the literature is here attempted, but sufficient data have been presented to indicate that many groups of trematodes which manifest marked similarity in structure and development have members, some of which infest marine and others which infest fresh-water hosts. The agreement in morphology and manner of development, recurring so consistently in different groups, can hardly be fortuitous, and the majority of investigators are agreed that these groups are formed by closely related rather than convergent species.

If, as has been postulated, these groups contain closely related species, the question naturally arises as to whether the common ancestral form occurred in marine or fresh-water hosts and how the present distribution was effected. A factor which must be kept in mind throughout the discussion is the complicated life history of these digenetic forms. Typically, sexual multiplication occurs in a vertebrate host, and eggs are produced which pass from the body of the host. From the eggs there emerge aquatic, ciliated larvæ which invade the first intermediate host,

always an invertebrate and usually a mollusk, where asexual multiplication takes place. A second type of aquatic, tailed larvæ leaves the first intermediate host, and these larvæ, either by direct penetration or after encystment on aquatic plants or in the bodies of other intermediate hosts, finally reach the vertebrate host. The free-living, larval stages are extremely delicate, ephemeral, and incapable of any extended migration. Consequently the presence of members of a common group in both marine and fresh-water hosts can only be explained by migration of the hosts or by transfer to new hosts.

The migration of free-living species from a marine to a fresh-water habitat, or the reverse, is largely prevented by the physical, chemical, and biological factors that characterize the two types of environment. It is true that marine species have been cut off in arms of the ocean, *e.g.*, the Caspian and Black Seas, which have subsequently become bodies of fresh water, and some of them have persisted although the number of such species is not large. Among the fishes, the anadromous and catadromous forms make regular migrations from one habitat to the other, but these examples stand as exceptions to the general rule. Fresh water imposes an effective barrier against the migration of Foraminifera, corals, echinoderms, cephalopods, and other groups of invertebrates. The relatively few invertebrates that have transferred from the ocean to fresh water have undergone extensive modifications in form and in life history. The free-swimming larval stages, characteristic of marine types, have almost entirely disappeared.

In an excellent study of this subject Needham (1930) has discussed the factors which prevent the penetration of marine organisms into fresh water. Allee (1923) has shown the effect of differences in temperature, oxygen content, and hydrogen ion concentration on the distribution of littoral invertebrates. Adolph (1925) studied certain physiological distinctions between fresh-water and marine organisms. He found that marine organisms show a much greater toleration for fresh water than fresh-water organisms do for sea water. His observations tend to support the long-accepted belief that organisms migrate from the ocean into fresh water, rather than in the opposite direction. In an interesting and suggestive study Marshall and Smith (1930) have attempted to correlate the composition of the body fluids of marine and fresh-water fishes with renal function and to trace the evolution and migration of these vertebrates on the basis of changes in the structure and activity of the excretory organs. Pantin (1931) has studied the triclad turbellarian *Gunda ulva*, which occurs in the estuaries of small streams, and found that these acelomate worms withstand both fresh and salt water, and that in nature they may be exposed to either extreme

for several hours. In tap water they double their volume and lose 25 per cent of their salt content in an hour. The presence of calcium reduces the rate of swelling and the loss of salts, presumably by reducing permeability. The significance of calcium and its relation to the problem of the migration of animals into fresh water was discussed.

The difficulties of migration from one habitat to the other would be greater in the case of parasitic species like the digenetic trematodes than in free-living forms. Where two or more hosts are involved, and where the transfer to the next host is effected by very delicate, short-lived, larval stages, the initial obstacles to migration are augmented by the difficulties inherent in the completion of the life cycle. For such a parasite to change from one location to another, either both primary and secondary hosts must have made the same migration simultaneously, or the parasite must have changed to new hosts as the migration progressed. Furthermore, and probably of greatest importance, the free-living, aquatic, larval stages must be able to withstand the changed environmental conditions and remain infective.

The migration of primary and secondary hosts has not been extensive in recent times at least. The groups of mollusks, fishes, reptiles, and mammals are clearly separated into marine and fresh-water species and this distinction has persisted with but very little change since earlier geological time. The shells of mollusks and skeletons of vertebrates afford suitable material for fossil formation and the geological history of several of these groups is known. According to Zittel (1913) "By means of analogy with recent species we are able in most cases readily to determine whether fossil forms pertain to land, fresh, brackish, or salt-water species." He stated that "not until the boundary between the Jura and Cretaceous is reached do we find any traces of fresh-water snails. . . . In the Wealden, and Cretaceous generally, both land and fresh-water gastropods are quite abundant; they become highly developed and widely distributed during the Tertiary, attaining, in fact, a differentiation nearly equal to that exhibited by the corresponding recent forms."

Since the vertebrate hosts are more active, wider ranging, and longer lived than the molluscan hosts, it would appear probable that if migration is to be considered as the explanation of present distribution, the vertebrate hosts were the principal migrants and that they were primarily responsible for change of habitat. The paleontology of the turtles (Hay, 1908; Williston, 1914) indicates that the marine turtles and the soft-shelled, fresh-water turtles have been separate, independent groups since the Mesozoic era. Looss (1902) described several genera of pronoccephalid trematodes from marine turtles; Stunkard (1930) and Mackin (1930) have described members of the same family from the

fresh-water turtles, *Amyda* and *Pseudemys*. The discovery of related parasites in hosts that have been separated since the Mesozoic would suggest that migration of hosts is not to be accepted as an explanation of these cases at least.

There are also serious objections to the explanation involving transfer to new hosts. While host parasite specificity is not so limited as was formerly believed, and it is well known that many parasites may infest several host species, as a rule the possible host species are closely related. For this hypothesis it is essential also that both old and new hosts live in the same habitat, since otherwise they would never encounter the infective larval stages of the parasite. Consequently, if separation into marine and fresh-water species was effected by the adoption of new hosts, the transfer could occur only in those regions where fresh and salt-water habitats overlap, namely at the mouths of rivers. In the transitional zone of brackish water, with the recurrent increase and decrease of the salt content and pH of the water with the rise and fall of the tide, transfer to new hosts may have caused divergence into definitely marine and fresh-water species.

It is thus possible that both migration of hosts and transfer to new hosts or a combination of the two methods may have been operative in producing present distribution of related species. It may be that the distribution of existing groups of digenetic trematodes is correlated with the origin of these groups and this point should be considered in any treatment of the problem. The present complicated developmental cycles could not have been the original or primitive life histories of these species. It has long been recognized that parasites have been derived from free-living progenitors. Competent investigators agree that the trematodes and cestodes have a turbellarian ancestry. The subject was discussed by Meixner (1926) with the following summary, "Es ergeben sich drei Schlüsse:

" I. Dass die Differenzierung der Trematoden und Cestoden mit dem Auftreten der Wirbeltiere eng verknüpft ist.

" II. Dass die zum Parasitismus auf Evertebraten übergegangenen Vorfahren der Digenea und Cestoden entsprechend der heutigen Beschränkung der primären Larven bereits auf verschiedene Wirtstierklassen spezialisiert waren.

" III. Trematoden und Cestoden sind zwei infolge ihres Parasitierens auf Wirbeltieren insbesondere hinsichtlich des Integumentes der Reifestadien abgeänderte Anhangsgruppen der Rhabdocoela."

Concerning the origin of these groups Reisinger (1928) stated, "Bezeichnend für die Amara ist die in vielen Gruppen vorherrschende Neigung zu parasitärer Lebensweise, vielleicht in Ausnützung einer

besonderen, dem ganzen Unterstamm eigenen, stoffwechselphysiologischen Konstitution, die den einzelnen Gruppen den Übergang zu intramolekularer Atmung (Glykogenabbau) besonders erleichterte. Die Urheimat der *Amera* ist zweifellos das Meer; der Übergang zu terrikoler und parasitischer Lebensweise mag sowohl von dort aus wie auch vom Süßwasser erfolgt sein und erfolgen."

Bresslau and Reisinger (1928) concluded that, "Unter den Rhabdocoelen verdienen die Familien der Graffilliden und Anoplodiiden besonderes Interesse, insofern als von ihnen aus vermutlich die Entwicklung der Trematoden ihren Ausgang genommen hat. Nach ihrer ganzen Organization sind die Monogenea wahrscheinlich von Graffilliden, die Digenea von Anoplodiiden oder anoplodiidenähnlichen Kalyptorhynchiern (Rhabdocoela) herzuleiten. Gut stimmt damit überein, dass gerade diese Familien das Hauptkontingent an Parasiten unter den Strudelwürmer stellen."

Consideration of this subject raises one of the most difficult problems in biology, the origin of intermediate hosts and the digenetic life cycle. The original ancestors of the digenetic trematodes must have become parasites of aquatic animals and the evidence indicates that mollusks were the original hosts. The type of reproduction in these mollusks is problematical. It is well established that parasitism increases reproductive activity, that it leads to new and accessory methods of reproduction, and that asexual multiplication is frequently interpolated between the sexual phases. There may have been a sexually mature, free-living stage after asexual multiplication was developed in the invertebrate host. The appearance of the vertebrate host may be correlated with the evolution of vertebrates and their use of mollusks as food.

Presumably the adoption of the parasitic habit occurred at an extremely remote period and the evolution of parasitic life histories and accompanying transformation of the parasites have proceeded hand in hand with the evolution of their hosts. The parallel evolution of hosts and parasites has been demonstrated by many authors. The presence of related species in both marine and fresh-water hosts may be explained by assuming that the primitive hosts harbored the ancestors of present species, and that the hosts have subsequently separated and differentiated into marine and fresh-water species. Such an explanation would imply that descendants of the original hosts have carried their parasites with them since the separation and, as a result of the ensuing migration and modification, the primary hosts, secondary hosts, and parasitic species have evolved together. It may be contended that this explanation merely pushes the problem further back in the time scale, renders it



more difficult of analysis, and less susceptible of experimental treatment. While to a degree this criticism is valid, the postulate may nevertheless be correct, and there are, moreover, experimental means of investigating the question.

Studies of much modified parasitic species and interpretation of their life histories are greatly facilitated if the life cycle contains free-living larval stages. These stages presumably correspond to ancestral ones, since it is generally true that embryonic and early developmental stages are very conservative and tend to remain unchanged regardless of modifications which may occur in the later development of the animal concerned. Since digenetic trematodes have such free-living larval stages, and since these larvæ are the infective agents, providing for the transfer from one host to another,—an experimental study is possible. Knowledge concerning the effects of environmental changes on these larvæ may have significant value in the interpretation of life cycles and distribution. Since the trematodes have two free-living larval stages in the life cycle, data should be obtained for both the miracidial and cercarial stages. It is often difficult if not impossible to secure miracidia in sufficient numbers for such experiments, while cercariæ can usually be obtained in abundance. It therefore seemed pertinent to make a study of the effect of the dilution of sea water on the activity and longevity of marine cercariæ. A corresponding study, already started, on the effects of diluted sea water on fresh-water cercariæ will give data, which, correlated with those from the present investigation, may aid materially in explaining present distribution of related species in marine and fresh-water hosts.

No matter whether the present distribution is explained through migration of original hosts or transfer to new hosts, the essential factor involved is the ability of the free-swimming larvæ to live and remain infective in the new environment. The ability of these larval stages to function in increasing or decreasing salinity indicates the direction of migration and the original home of the original trematode species. Consequently, the experiments reported in the present paper were undertaken.

#### MATERIAL AND METHODS

All of the cercariæ used in the investigation were obtained from mollusks of the Woods Hole region, and the experiments were done at the Marine Biological Laboratory during the summer of 1930. Data are given in the tables for the following six species: (1) the cercaria of *Cryptocotyle lingua* from *Littorina littorea*, (2) *Cercariæum lintoni* from *Nassa obsolcta*, (3) *Cercaria quissetensis* from *N. obsolcta*, (4) *C. variglandis* from *N. obsolcta*, (5) *C. parvicaudata* from *L. littorea*, and (6) *C. sensifera* from *Urosalpinx cinereus*.

The snails were isolated in small dishes of sea water to determine those from which cercariæ were emerging. Several of those infected by one and the same species were then placed in a small dish for 10 to 12 hours in order to secure large numbers of recently emerged cercariæ. At the end of this period the snails were removed and the cercariæ transferred to small dishes of sea water, usually 20 to 50 in each dish. The sea water was removed from these dishes and replaced by sea water to which various amounts of tap water had been added. Solutions were made up as follows: (I) undiluted sea water; (II)  $\frac{3}{4}$  sea water,  $\frac{1}{4}$  tap water; (III)  $\frac{1}{2}$  sea water,  $\frac{1}{2}$  tap water; (IV)  $\frac{1}{4}$  sea water,  $\frac{3}{4}$  tap water; (V)  $\frac{1}{8}$  sea water,  $\frac{7}{8}$  tap water; (VI) tap water. The dishes were covered to prevent evaporation and kept at the temperature of the laboratory. In each experiment all of the larvæ were subjected to identical conditions except for the different amounts of tap water in the solutions. The only variable factor, therefore, was the amount of tap water and the results show the effects of increasing dilutions of sea water. Observations were made with a binocular microscope at appropriate intervals and the condition and activity of the larvæ noted.

*Cercaria of Cryptocotyle lingua*

An abstract of this experiment was reported (Stunkard, 1930c).

At first the larvæ are very active and all swim vigorously by rapid lashing of their tails, holding the body motionless in a curved position. Swimming movements cause the cercariæ to rise toward the surface of the water and when swimming is temporarily suspended the larvæ slowly sink. They are positively phototropic and accumulate at the light side of the dish. As the vitality of the larvæ diminishes they become progressively weaker and are unable to rise from the bottom. This is due primarily to exhaustion of the tail muscles. The larvæ then extend and retract their bodies and tails, although since there is no functional acetabulum they can make little forward progress. In the solutions which contain 50 per cent or more of tap water the tails begin to swell and lose their motility, and later the body swells. Naturally the swelling is more rapid and greater in the more dilute solutions. Apparently swelling is inhibited so long as the tissues are alive and active. The swelling causes cytolysis of the tails and they soon drop off. The tails are frequently lost after a few hours in all of the solutions. In the tables the following notations are used:

Swimming vigorously + + + +  
 Swimming seldom and feebly + + +  
 Contracting vigorously ++  
 Contracting feebly +  
 Dead —



The experiments were repeated four times and the results are in substantial agreement. The following protocol is representative.

Solutions.....	I	II	III	IV	V	VI
No. of cerearie Aug. 4 Time	25	69	53	70	140	172
2:00 P.M.	25	69	53	70	140	172
2:20	all + + + + +	all + + + + +	all + + + + +	all + + + + +	all + + + + +	80 + + 92 + +
2:30	all + + + + +	all + + + + +	all + + + + +	all + + + + +	all + + + + +	86 + + 86 -
3:00	all + + + + +	all + + + + +	all + + + + +	68 + + + + + 2 + + + + +	50 + + + + + 90 + + + + +	1 + 171 -
4:00	all + + + + +	all + + + + +	all + + + + +	65 + + + + + + + + + + 1 + + +	140 + +	
6:00	23 + + + + + 1 + + + + + 1 + + + + +	60 + + + + + 5 + + + + + 2 + + + + + 2 - - - - -	44 + + + + + 7 + + + + + 1 + + + + + 1 - - - - -	58 + + + + + 11 + + + + + 1 + + + + +	6 + + + + + 4 + + + + + 32 + + + + + 10 + + + + + 88 - - - - -	

Solutions.....	I	II	III	IV	V	VI
No. of cercariae..... Aug. 4 Time	25	69	53	70	140	172
8:00 P. M.	19++++ 3++++ 3+++	57++++ 8++++ 2++ 2--	33++++ 18++++ 1+++ 1--	56+++++ 13++++ 1+++	7++++ 35++++ or + 97--	
Aug. 5 9:00	8+++++ 15++++ 1+++ 1--	53+++++ 10++++ 6--	15+++++ 31++++ 6+++ 1--	21+++++ 30++++ 7+++ 5--	10+ 130--	
11:15	8+++++ 14+++ 3--	41+++++ 20+++ 8--	14++++ 36+++ 3--	15+++++ 50+++ 5--	5+ 135--	
1:15	22+++ 3--	35+++++ 26+++ or + 8--	50+++ or + 3--	5+++++ 60+++ or + 5--	5+ 135--	
3:15	22+++ 3--	25++++ or ++ 36+++ or + 8--	50+++ or + 3--	5+++++ 60+++ or + 5--	2+ 138--	
Aug. 6 9:00	4+ 21--	5+ 64--	53--	70--	140--	

Analysis of the data shows that Solutions II, III, and IV have only slightly harmful effects as the sea water is diluted. In solutions containing 50 per cent or more of sea water the effect is not significant and, in two of the experiments, after 12 hours in Solution No. II the cercariae were more active and vigorous than those in undiluted sea water. In Solution No. V, which contained  $\frac{1}{8}$  sea water, the cercariae were all on the bottom of the container at the end of 2 hours. They had begun to swell noticeably, some had lost their tails, and those whose tails beat rapidly were unable to rise in the water. It is probable that the larvæ are not infective in this concentration for more than a few minutes. The range between  $\frac{1}{8}$  and  $\frac{1}{4}$  sea water appears to be the critical zone where the dilution of the sea water exerts a markedly harmful effect on the physiological processes of the larvæ. Freshly emerged cercariae manifest normal swimming movements for only a few minutes when placed in tap water; at the end of 20 minutes all had settled to the bottom, in 30 minutes about  $\frac{1}{2}$  of them showed no sign of life and the others soon succumbed.

*Cercaricum lintoni* Miller and Northrup, 1926

Solutions were made up as in the previous experiment and the same procedure was followed. Since these larva have no tails they can not swim and their activity is restricted to creeping movements as described by Miller and Northrup (1926). The experiment was repeated seven times using 20 recently emerged cercariae in each dish. In two of the tests distilled water was used instead of tap water and the larvæ lived as long, and in certain of the dishes slightly longer than in those containing the same amount of sea water diluted with tap water. The differences were not great and probably are not significant. The results are similar for all experiments and the following protocol, given on p. 253, is typical.

These results are similar to those obtained for the cercariae of *C. lingua*. The larvæ show very little normal activity after 15 minutes in tap water and it is apparent that they are not infective in this medium. Two larvæ encysted on the bottom of the dish but it is apparent that encystment in the water is not a usual or normal stage in the life history of the species.

*Cercaria quissetensis* Miller and Northrup, 1926

The experiments were conducted as previously described and repeated eight times, using 20 cercariae in each dish. In swimming, the

Solutions.....	I	II	III	IV	V	VI
No. of cercariæ.....	20	20	20	20	20	20
Time in Hours						
1	20++	20++	20++	20++	20++ or +	20-
2½	20++	20++	20++	20++	20++ or +	
6	20++	20++	20++	20++	18++ or + 2-	
12	20++	20++	20++	20++	13+ 7-	
24	17++ or + 3+ or -	16++ or + 4+ or -	16++ or + 4+ or -	15++ or + 5+ or -	12+ 8+ or -	
36	15++ or + 5+ or -	14++ or + 6+ or -	15++ or + 5+ or -	12++ or + 8+ or -	7+ 13+ or -	
48	11++ or + 9-	5+ 15+ or -	4++ or + 16+ or -	4++ or + 16+ or -	1+ 19-	
60	3+ 17-	2+ 18+ or -	2+ 18+ or -	1+ 19+ or -	20-	
70	1+ 19-	20-	1+ 2+ or - 17-	1+ 2+ or - 17-	20-	

body assumes a spherical form and the tail lashes vigorously. Records were taken every hour and the following protocol, given on pp. 255 and 256, tabulates the results of one experiment.

In this species the cercariæ lose motility after 15 to 30 minutes in tap water. The tails swell in all the solutions containing 50 per cent or more of tap water and soon become detached. There is a tendency for the cercariæ to encyst after 24 hours in the more concentrated solutions and such larvæ removed from their cysts at the end of 72 hours were alive and active. There were only slight differences between the cercariæ placed in Solutions I and II. During the first half of the experiment the larvæ in Solutions III and IV appeared to be affected more than those in Solutions I and II, but in three of the tests they lived longer than those in undiluted or 75 per cent sea water.

*Cercaria variglandis* Miller and Northup, 1926

This species is very rare. Miller and Northup found only 3 infested snails among 8,875 individuals of *Nassa* examined, and we found only 2 infected snails. The structure of the cercaria indicates that it is the larva of one of the blood flukes, and it swims in active spurts. Only two experiments were made, but the results, given in the protocol on p. 257, indicate that the larvæ are short-lived and very delicate. The procedure was the same as that previously employed.

As the cercariæ lose motility they become distorted, the furcæ coil up and it is sometimes difficult to determine whether or not they are dead. Soon, however, they turn dark-colored and later they tend to float.

*Cercaria parvicaudata* n.sp.

Two experiments were made with these larvæ. The procedure was the same as that previously employed, although the observations were not continued until the death of the cercariæ. One of the protocols is given on p. 258 and the other is in essential agreement.

The cercariæ in Solution VI (tap water) were all dead and their bodies much swollen at the end of one hour. At this time all of those in Solutions I, II, and III were swimming intermittently, while those in Solutions IV and V were unable to leave the bottom. They were all lying on the dorsal side, bodies bent as in swimming, with the tails moving. After four or five hours they seemed to be more active although they were unable to leave the bottom of the container. At the end of 25 hours, although they were unable to swim, the larvæ in Solution II were more active than those in sea water, and those in 50 per cent sea water were more active than those in Solution II.

Solutions.....	I	II	III	IV	V	VI
No. of cercariæ.....	20	20	20	20	20	20
Time in Hours						
1	20+++++	20+++++	20+++++	20+++++ or +++++	20+++++ or +++++	20-
2	20+++++	20+++++	20+++++	20+++++ or +++++	3++++ 17++++	
4	20+++++	20+++++	20+++++	20+++++ or +++++	2++++ 18++++	
6	20+++++	20+++++	20+++++	18+++++ or +++++	2++++ 18++++ 8 tailless	
12	19+++++ or 1+++ (tailless)	18+++++ or 2+++ (tailless)	8++++ or 12+++ 9 tailless	7++++ or 13+++ 11 tailless	2++++ 18++++ or 15 tailless	
24	12++++ or 7+++ 1 cyst 8 tailless	8++++ or 10+++ 2-	4++++ or 13+++ or 2 cysts 1-	5++++ or 15+++ or	1++++ or 6-	

Solutions.....	I	II	III	IV	V	VI
No. of cercaria.....	20	20	20	20	20	20
Time in Hours						
36	7++ or + 2 cysts 6+ or - 5-	5++ or + 9+ or - 6-	13++ or + 2 cysts 5-	11++ or + 3+ or - 6-	8++ or + 3+ or - 9-	
48	5++ 2 cysts 13-	6++ or + 14-	7++ or + 2+ or - 9- 2 cysts	6++ or + 8+ or - 8-	5++ or + 3+ or - 12-	
60	2+ or - 2 cysts 10-	2+ or - 18-	3+ 2 cysts 15-	4+ 1 cyst 15-	20-	
70	2 cysts 18-	20-	2 cysts 18-	1+ 1 cyst 18-		

Solutions.....	I	II	III	IV	V	VI
No. of cercariae.....	20	20	20	20	20	20
Time in Hours						
1	20+++++	20+++++	20+++++	19+++++ 1+++	15+++++ or +++++	12+ 8-
2	20+++++	20+++++	19+++++ or 1+++	17+++++ or 3++++ or +++	12+++++ 8+++	6+ 1+-
6	14+++++ or 6+++++ or +++	17+++++ or 3+++	15+++++ or 5+++	11+++++ 4+++ 5--	6+ 14--	20-
12	4+++++ 12+++ 4-	7+++ 5+ 8-	5+++ 3+ 12--	4+++ or + 6+ or - 10-	20--	
24	20--	20--	2+ or - 18-	2+ 18-		



Solutions.....	I	II	III	IV	V	VI
No. of cercaria.....	39	65	60	80	90	100
Time in Hours						
1	39 + + + + +	65 + + + + +	60 + + + + +	80 + +	90 + +	100 -
6	39 + + + + +	65 + + + + +	60 + + + + +	80 + +	90 + +	
	39 + + + + + or + + + + +	65 + + + + + or + + + + +	60 + + + + + or + + + + +	80 + +	38 + + 45 + + 7 -	
25	32 + + or + + 7 -	65 + + or + +	59 + + or + + 1 -	15 + + 55 + +	15 + + 47 + + 28 -	

*Cercaria sensifera n.sp.*

Four experiments were performed with these larvæ, and the results of one experiment are tabulated in the following protocol, given on p. 260.

There is a pronounced tendency for these cercariæ to encyst when subjected to unfavorable conditions. The process of encystment is rapid and the cysts are either free or attached to the bottom of the container. Presumably this phenomenon is normal and significant for life history studies of the species.

Cercariæ emerge usually at night or in the early morning and the majority soon encyst. They tend to adhere to any object they touch and numbers stick to the inside of a pipette used to transfer them from one solution to another. After attachment they soon encyst.

## DISCUSSION

In an investigation of this character, it is desirable to study as many species as possible and representatives of different taxonomic groups. Unfortunately, information concerning the marine larval trematodes of North America is very meager. Only a random sample of the species has been described. The literature dealing with these larvæ was reviewed by Miller and Northup (1926), who described five species from *Nassa obsoleta* at Woods Hole, Massachusetts. It is significant that only one of the five had previously been reported. Since these were almost the only larval trematodes described from the Woods Hole region, an attempt was made to secure them for the present study. All of the five species described by Miller and Northup were found and three of them in sufficient numbers for the experiments. Of the other three species studied, one was shown by Stunkard (1930a) to be the larva of *Cryptocotyle lingua*, while the two remaining species are new to science and are described in a later section of this paper.

For these experiments it is essential that cercariæ be available in large numbers. Since cercariæ secured by crushing parasitized snails are immature and not infective (Stunkard, 1930, 1930b), such larvæ do not constitute suitable material, and results obtained from them are probably not significant. Consequently, only normally emerged cercariæ were used. Since several hours are required for the emergence of sufficient numbers, some of the cercariæ had been swimming for ten to twelve hours when the experiments were started. This factor undoubtedly accounts for much of the variation shown in the results. Presumably the most recently emerged cercaria lived the longest.

It is apparent in all species studied that tap water exerts an immediate and harmful effect. None of the cercariæ showed normal

Solutions.....	I	II	III	IV	V	VI
No. of cercariae.....	11	17	19	22	20	20
Time in Hours						
1	14 + + + + +	17 + + + + +	19 + + + + +	18 + + + + + 4 encysted	12 + 8 -	20 -
3	13 + + + + + 1 + + (tailless)	17 + + + + +	18 + + + + + 1 + + (tailless)	14 + + + + + 2 + + 4 encysted 2 -	20 -	
6	12 + + + + + 1 + + 1 encysted	17 + + + + +	15 + + + + + 3 + + 1 encysted	6 + 6 encysted 10 -		
15	6 + + + + + 8 encysted	16 + + + + + 1 encysted	15 + + + + + 3 encysted 1 +	2 + 6 encysted 14 -		
22	3 + + 8 encysted 3 -	9 + + + + + 8 encysted	4 + + 14 encysted 1 +	2 + 6 encysted 14 -		
39	8 encysted 6 -	11 encysted 6 -	15 encysted 4 -	6 encysted 18 -		

activity for more than a few minutes and most of them died within an hour. The bodies and tails became swollen, the tissues underwent cytolysis with the absorption of water, and death followed shortly. Presumably there was a diffusion of salt from the organisms as water was absorbed and the loss of salt would augment and hasten the deleterious effects produced by the imbibition of water. Obviously these cercariæ can be infective for only a very brief period in tap water and it is doubtful whether they could complete their life cycle in fresh water, even if suitable hosts were available.

Larvæ placed in Solution V, containing seven-eighths tap water and one-eighth sea water, were active for considerable periods of time and some of them were able to perform swimming movements for one to four hours. Certain of them, *e.g.*, *Cercaria parvicaudata*, appear to be deleteriously affected after a short time in this concentration, and later they partially recover. They may continue to live for several hours, although the earlier ill effects are not entirely remedied and it is doubtful whether such larvæ would be infective. They are not infective in the case of *C. lingua* and since the life histories of the other species are unknown, experimental test is impossible.

The experiments show a marked difference in the activity and longevity of cercariæ in Solutions V and IV. Whereas a solution containing one-eighth sea water is definitely harmful, cercariæ manifest little in the way of ill effects in solutions containing 25 per cent sea water. In one-fourth sea water the larvæ live almost as long as in greater concentrations, although they are usually less active after the first few hours. The sluggishness may be due to the increased water content. Cercariæ may succumb somewhat more quickly in one-fourth sea water than in more concentrated solutions, but they are normal in appearance and activity for sufficiently long periods of time to indicate that they may be infective and able to function normally in continuing the life cycle.

Considerable interest attaches to the observation that larvæ are active and apparently normal for almost if not quite as long in solutions containing 50 per cent or more of sea water as they are in undiluted sea water. In certain experiments, cercariæ actually lived longer in one-half than in undiluted sea water, although they were not normally active and probably not infective for longer periods than larvæ in sea water.

The ability of cercariæ to withstand dilution of sea water is roughly proportional to the dilution which occurs in the larger bays. Cowles (1930) reported that, "The salinity of Chesapeake Bay, like that of other long bays and estuaries, gradually decreases, with very few exceptions, from the mouth to the head; and the bay is known as a

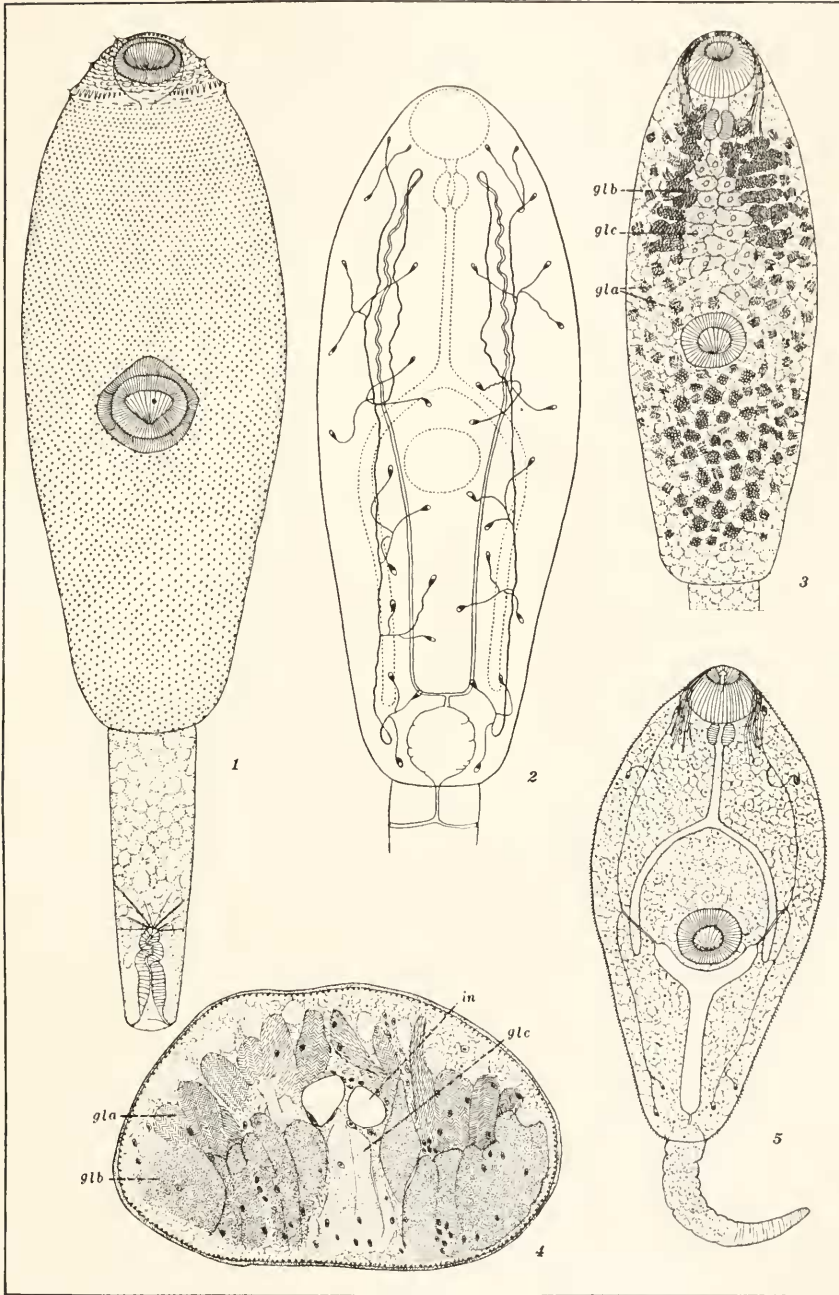
brackish body of water, although the failure as a rule, of the fresh waters from the land and the saline waters of the sea to mix completely, and the variation in the volume of fresh and salt water entering the bay, result in different degrees of brackishness. The surface data at the mouth of the bay show a variation in salinity from about 19 to 30 grams per liter, while near Baltimore there is a variation from about 3 to 11 grams per liter." . . . "The bottom salinities recorded on our cruises for the mouth of the bay varied from about 26 to a little over 32, while in the region of Baltimore they varied from about 6 to 17."

It appears more than probable that such transitional zones, extending sometimes for a distance of a hundred miles or more, with ocean water at one end and fresh water at the other, provide the ecological settings in which species become transformed physiologically and structurally from marine to fresh-water organisms, and vice versa. Due to the well-known and constantly appearing variations which occur among animals, certain fresh-water species may have become adapted to life in brackish and eventually sea water, while similarly, marine species may have entered fresh-water habitats.

If the larvæ of parasitic species are able to survive in a new and different environment long enough to find and infect a suitable host, either a former host that has migrated into the location, or a new host species, the life cycle may be completed. The long list of snails which serve as intermediate hosts of *Fasciola hepatica*, under different conditions in various parts of the world, demonstrates the extent to which that species has acquired new hosts and the work of Cort (1918) illustrates the ability of other trematode larvæ to successfully attack new hosts. In the case of a marine or brackish-water species entering fresh water, the essential factor is the ability of the free-swimming larvæ to withstand the hypotonic medium until infection is accomplished, since in the body of the host a medium of higher salt content and tonicity is encountered.

The present experiments record the ability of six marine cercariæ to withstand dilution of sea water and show that these larvæ manifest normal activity for considerable periods of time in solutions containing only one-eighth to one-fourth sea water. The observations indicate that these cercariæ are able to complete their life cycles in brackish water and denote the extent to which marine species may migrate into brackish and fresh water. The paper thus contributes toward the solution of the problem of the distribution of related species in marine and fresh-water habitats.

## PLATE I

FIG. 1. *C. sensifera*, ventral view.FIG. 2. *C. sensifera*, excretory system.FIG. 3. *C. sensifera*, ventral view, showing distribution of gland cells.FIG. 4. *C. sensifera*, cross section, showing three types of gland cells.FIG. 5. *C. parvicaudata*, ventral view.

*Cercaria parvicaudata* n.sp.

(Fig. 5)

This species occurs in about one per cent of the specimens of *Littorina littorea* examined at Woods Hole. The cercariae are produced in sporocysts which occupy the lymph spaces of the snail. The gonad is the principal seat of infestation and frequently this organ is entirely destroyed.

The cercariae are small, with oval to pyriform bodies and short tails. The length of the body varies from 0.14 to 0.36 mm. in contracted and extended condition. The tail is very active; it may be contracted to a length of 0.06 mm. or extended until it exceeds the body in length. Ordinarily when the worm is attached or creeping, the tail is contracted and manifests a nervous, twitchy motion. In swimming the body is contracted into a short, wide form, bent ventrally and the tail is extended and lashes violently.

The body is covered with minute spines and the oral sucker bears a stylet 0.015 mm. long by 0.0032 mm. wide. There is a small thickening on the stylet near its tip. The acetabulum is situated near the middle of the ventral surface and measures from 0.03 to 0.05 mm. in diameter.

The cercariae encyst readily. A portion of a dissected snail was left for six hours in a watch glass and ten cercariae had encysted among the sporocysts. Other cysts were found in the tissue of the snail which had been fixed and later sectioned. The cysts measure approximately 0.17 mm. in diameter. There are gland cells of two types distributed throughout the parenchyma of the body; one is filled with refractive, spherical granules, the other is slightly opaque and contains very fine granules. On either side of the mouth there are openings of ducts which pass backward and appear to communicate with other glandular cells situated in the preacetabular region, although the connections of these ducts were not determined with certainty. Presumably they are the ducts of penetration or salivary glands.

The oral sucker is spherical to oval, 0.035 to 0.06 mm. in diameter. It is followed almost immediately by a small pharynx and the esophagus extends about one-half of the distance to the acetabulum. The digestive ceca terminate blindly near the level of the caudal margin of the acetabulum.

The excretory vesicle is Y-shaped, with a long stem and short branches. Its wall contains large, deeply-staining cells. Four flame cells have been definitely located and these are shown in the figure. Others were observed, but their connections were not traced. The reproductive organs are represented by a mass of cells which are dorsal and anterior to the acetabulum.

This species belongs in the large and heterogeneous group of Niphidiocercarize, but further attempts to relate it must await a more complete knowledge of its morphology or information concerning its adult form.

*Cercaria sensifera* n.sp.

(Figs. 1-4)

This species has been found only in the oyster drill, *Urosalpinx cinereus*, and it was present in fourteen out of 594 specimens examined during the summer of 1930. Six infestations were found in two hundred and six snails collected at Woods Hole during the first week of April, 1931. The parasites infest the interlobular areas of both the reproductive and digestive glands. In an uninfected snail the visceral mass is plump, the liver is yellow and the gonad is cream-colored, whereas in a parasitized snail the organs are shrunken, the gonad may be destroyed, and the body is lighter in color.

The cercariæ (Fig. 1) are large and clearly visible to the unaided eye. The body is oval in shape, more or less elongated and narrower posteriorly, flattened dorsoventrally, and widest in the preacetabular region. It is truncated posteriorly and the attachment of the tail is terminal. The cercariæ vary considerably in size and manifest much elongation and contraction in locomotion. They are not active swimmers and tend to remain near the bottom of the water. After a time swimming movements alternate with creeping ones. In swimming, the tail is elongated; it does not lash about, but the cercaria moves by undulatory movements of the body and tail. The chief propulsive force comes from the anterior half of the body. It slowly bends ventrally and then snaps backward, pulling the larva forward. The movement is continued through the posterior part of the body and tail producing the sinuous motion of the larva.

With the exception of the anterior end, the body is covered with a thick granular cuticula which bears large, closely set spines. Those in the anterior row are considerably larger than the others. This row is interrupted in the midventral region. There are 44-48 spines on the dorsal side and 10 on each side ventrally. These spines measure 0.005-0.006 mm. in length. This region of the body is sometimes contracted to produce a distinct collar-like effect. There are about forty-five annular rows of spines in the preacetabular region and about 130 to 135 such rows on the body. The spines in successive rows alternate with each other and those around the acetabulum are arranged in concentric rings. The cuticula of the tail is thin and smooth.

The larvæ are bottom forms, and attach readily to any available



surface. When picked up in a pipette they frequently adhere to the inside of the tube and can be dislodged only with great difficulty. They may become attached either by the suckers or by the tip of the tail and after attachment they soon encyst. If placed in solutions that are irritating, *e.g.*, too strong concentrations of vital dyes, they encyst almost immediately. The cyst consists of two layers, a thick, opaque, external covering and a thin, transparent, very tough, inner membranous layer.

Neither of the cyst walls is readily stainable by ordinary dyes. The cyst is oval, flattened on the side of attachment, and measures from 0.2 to 0.23 mm. in width by 0.23 to 0.27 mm. in length. The tail is always detached in encystment and may remain attached for a time to the surface of the cyst. Normally the worm fills the cyst completely. The outer cyst wall is easily removed by rolling a cyst between a slide and cover glass, but it is difficult to get the worm out of the inner membranous covering without injury.

Living cercariæ may extend to a length of 0.9 mm. and contract until the length is no greater than the breadth. The tail also is capable of much extension and contraction; it may be very much shortened or elongated to almost the length of the body. In the latter condition it is slender with an expanded, cup-shaped portion at the end. The caudal tip is usually introverted in a characteristic manner (Fig. 1), although the invaginated portion may be protruded and apparently bears a sticky substance, by means of which the cercariæ adhere to objects. Fixed and stained specimens measure from 0.21 to 0.47 mm. in length and 0.14 to 0.26 mm. in width. In such specimens the tails vary from 0.12 to 0.26 mm. in length. The acetabulum is situated slightly behind the middle of the body. In living specimens it measures from 0.08 to 0.1 mm. in length and from 0.1 to 0.115 mm. in width, while in fixed and stained specimens it measures from 0.68 to 0.76 mm. in diameter.

The anterior end of the body is covered by a smooth, thin, unarmed cuticula which extends backward as far as the caudal margin of the oral sucker. This region bears a number of papillæ, arranged irregularly in two or three rows, and each papilla terminates in a bristle. Similar structures occur around the margin of the acetabulum and presumably they function in a sensory manner. This idea is expressed in the specific name, *sensifera*. The caudal one-fourth or one-fifth of the anterior unarmed area forms a zone which frequently is marked by small longitudinal furrows.

The body is filled with gland cells of several types. In the region between the pharynx and acetabulum there are twelve large unicellular glands. These cells (Figs. 3, 4, *glc*) have very small secretory granules and open to the surface through twelve pores situated at the anterior tip

of the body above the oral sucker. They seem to correspond to penetration glands of other cercariæ and indicate that the larvæ at some later stage bore into the tissues of an intermediate host. The cortical layer of the parenchyma contains numerous dermal glands. In addition, the dorsal half of the body contains numerous gland cells (Figs. 3, 4, *gla*), the cytoplasm of which is filled with bacilliform granules or rods. These cells do not stain with neutral red and in sections counterstained with erythrosin the secretory products appear yellowish. The ventral portion of the body is largely filled with gland cells (Figs. 3, 4, *glb*) whose cytoplasm contains large spherical secretory granules. The cell contents stain intensely with erythrosin. In the anterior half of the body these cells appear to be arranged in four longitudinal fields, separated by the large ventrolateral nerve trunks and the esophagus.

Attempts were made to study the cercariæ in solutions of various vital dyes. With neutral red the spine-covered portion of the cuticula and the cells which secrete it quickly take up the stain and this red or pink layer at the surface of the body masks the action of the stain inside. It is clear, however, that the contents of the digestive ceca assume a deep red color. The stem of the tail, with the exception of the caudal third or fourth, is filled with large fluid globules that take the stain and become a brick-red color. Young specimens do not take the stain at all, and at a later stage the bodies of the larvæ assume a diffuse pink or rose color. The use of other stains, methylene blue, dahlia, pyronin, brilliant cresyl blue, Janus green, light green, and methyl violet did not give significant results on this cercaria. If very dilute solutions were employed the staining was slight and diffuse, not differential, and when stronger solutions were used the cercariæ encysted very quickly.

The mouth opening is subterminal and the oral sucker measures from 0.06 to 0.08 mm. in diameter in living specimens. In fixed and stained specimens the diameter is from 0.05 to 0.06 mm. There is a short prepharynx, the pharynx measures from 0.02 to 0.027 mm. in diameter, and the esophagus is long, extending about two-thirds of the distance to the acetabulum, where it bifurcates to form the intestinal ceca (Fig. 4, *in*). The ceca end blindly about two-thirds of the distance from the acetabulum to the caudal end of the body. The esophagus as well as the ceca is lined with epithelium and this point is significant for life history and taxonomic identification.

The excretory system (Fig. 2) has been worked out in detail and the pattern confirmed on dozens of cercariæ. The system forms as two separate parts, one right and the other left, as described for other cercariæ. The longitudinal ducts fuse near the posterior end of the body and this portion becomes the future excretory vesicle. With the con-

striction that forms the tail the posterior end of the vesicle is denoted and the two excretory pores open on either side of the tail as shown in Fig. 2. The details of the excretory system are shown in the figure. The collecting ducts extend forward to the level between the pharynx and oral sucker, passing on the ventral side of the intestinal caeca. The anterior portions of these ducts contain excretory concretions. Each collecting duct turns posteriorly and this recurrent stem contains two large ciliated areas. At the level of the intestinal bifurcation the recurrent ducts divide into anterior and posterior branches. Each of the anterior and posterior branches bears three clusters of flame cells with three cells in each cluster. The cone of cilia in a flame cell measures from 0.008 to 0.01 mm. in length.

The reproductive organs are represented by a mass of deeply staining cells, the anlagen of the gonads, situated in front of the excretory vesicle, and a strand of cells which extends forward connecting with another cell mass in front of the acetabulum. The strand of cells will form the gonoducts and the cell mass in front and sometimes to the left of the acetabulum is the anlage of the genital pore and copulatory organs.

The cercariae are produced in rediae. A redia has two "feet," a birth pore near the oral sucker, and an intestine which contains orange-colored granules. In a small redia the germ masses are situated in the caudal third of the body and the posterior tip may be protruded in a tail-like or foot-like protuberance that is used like the feet in locomotion. There is a muscular lip-like snout in front of the oral sucker; the sucker measures from 0.05 to 0.07 mm. in diameter and in a young redia the intestine extends through three-fourths of the body length. Rediae increase to a length of 2.1 mm. and a width of 0.4 mm. The small rediae may have one or more fully formed cercariae in their bodies and large rediae contain from ten to thirty more or less developed cercariae.

*Cercaria sensifera* belongs to the *Megalura* group of cercariae, outlined by Cort (1915) and extended by Sewell (1922). It agrees closely with *C. purpura* and *C. patella*, marine species described by Lebour (1907, 1912) and indeed may be specifically identical with *C. purpura*. Slight differences between the present specimens and the account of Lebour in regard to the arrangement of the gland cells, the presence of a "neck" region, and the relations of the excretory system and the tail, make it impossible to determine with certainty whether the specimens may be referred to *C. purpura*. Accordingly, a new name is proposed for them with the understanding that it will disappear as a synonym if further studies prove the American species to be identical with the European. In the paper cited, Lebour (1912) predicted that the adult stage of the parasite occurs in a bird. Two years later, Lebour (1914)

compared young stages of *Parorchis acanthus* Nicoll with *C. purpuræ* and identified the cercaria as the larval stage of that species, confirming a prediction made by Nicoll some years previously. Believing that *Parorchis* is closely related to the echinostomes, Lebour stated that the second intermediate host is probably a mollusk. In a later report, Lebour and Elmhirst (1922) reported that *C. purpuræ* encysted in the mantle of *Cardium edule* and *Mytilus edulis*. Their figure of the cyst from side view indicates that it is on the surface rather than embedded in the mantle of the host. The observations of Lebour and Elmhirst appear to complete the life cycle of the species but the experiments were not sufficiently controlled to exclude other possibilities. In the first and only successful experiment reported, the cercariæ used "swam actively by a strong side to side lashing of the tail." It is questionable whether these larvæ were actually *C. purpuræ* since megalurous cercariæ do not employ this method of swimming. Two types of larval trematodes were found encysted in the single specimen of *Cardium* used in the experiment and the more abundant species was identified as *Echinostomum secundum*. Consequently, two types of cercariæ were introduced unless the intermediate host was already infected when the experiment was begun. Apparently the authors did not know whether or not the bivalves used in the experiment were previously infected, and results of such experiments are not at all conclusive. If *C. purpuræ*, like *C. sensifera* will encyst on any surface to which it adheres, it is only natural that the larvæ should encyst on the mantle of mollusks placed in aquaria with them, and it does not necessarily follow that these mollusks are the normal intermediate hosts.

The idea at once presents itself that *C. sensifera* is a larval stage of *Parorchis acutus* Linton, 1914, an unusual trematode in which free miracidia were found in the uterus and in which each miracidium contained a well developed redia. Such a life cycle would explain the observation of Lebour that sporocysts or other preredia stages of *C. purpuræ* were not found in the snail host. Linton (1928) gave a further discussion of *P. acutus* and argued against the suggestion of Nicoll that the American species is identical with *Parorchis acanthus*.

#### SUMMARY

The problem concerning the origin and distribution of closely related parasites that occur in marine and fresh-water hosts is discussed. Migration of hosts and transfer to new hosts are the only explanations that appear tenable. The essential factor is the ability of the free-swimming larvæ to live and remain infective in a changed environment. Experiments on the ability of six species of marine cercariæ to withstand dilu-

tion of sea water show that these larvæ manifest normal activity for considerable periods of time in solutions containing only one-eighth to one-fourth sea water. The observations indicate that these cercariæ are able to complete their life cycles in brackish water and denote the extent to which these organisms may migrate into brackish or fresh water.

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